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CAS NUMBER	REPORT DATE	TITLE
101-68-8	October 8, 1991	Characterization of murine immune responses to allergenic diisocyanates

Sincerely,

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Supervisor, Product Safety

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Characterization of murine immune responses to allergenic diisocyanates

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ABBREVIATED TITLE : Immune responses to diisocyanates.

ABBREVIATIONS USED

AOO, 4:1 acetone:olive oil; BSA, bovine serum albumin; DNCB, 2,4-dinitrochlorobenzene; DTH, delayed-type hypersensitivity; GM-CSF, granulocyte/macrophage colony-stimulating factor; HMDI, dicyclohexylmethane-4,4′-diisocyanate; IFN-γ, interferon γ; IL, interleukin; IPDI, isophorone diisocyanate; LNC, lymph node cells; MDI, diphenylmethane-4,4′-diisocyanate; NGS, normal goat serum; TMA, trimellitic anhydride; TNBS, 2,4,6-trinitrobenzene sulfonic acid.

ABSTRACT

Characterization of murine immune responses to allergenic diisocyanates.
DEARMAN, R.J., SPENCE, L.M., AND KIMBER I. ( ). Toxicol. Appl. Pharmacol. Chemicals may cause contact allergy. Some allergens may, in addition, cause respiratory sensitization. In previous investigations we have found that contact and respiratory sensitizers induce differential immune responses in mice characteristic of T_{H1} and T_{H2} T helper cell activation respectively. In the present study we have examined immune responses in mice following topical exposure to 3 allergenic diisocyanates; diphenylmethane-4,4'-diisocyanate (MDI), dicyclohexylmethane-4,4'-diisocyanate (HMDI) and isophorone diisocyanate (IPDI). All three chemicals are contact allergens. MDI is in addition a known human respiratory allergen. HMDI and IPDI appear not to induce respiratory sensitization, or at least do so very rarely. Exposure of mice to all chemicals resulted in a vigorous lymphocyte proliferative response in lymph nodes draining the site of application, and each caused contact sensitization. In common with other respiratory allergens, MDI induced an increase in the serum concentration of IgE and provoked considerably more IgG2b than IgG2a anti-hapten antibody; responses consistent with a preferential activation of T_{H2} cells. In contrast, under conditions where both caused lymph node cell proliferation and contact sensitization, neither HMDI nor IPDI induced a measurable antibody response of any class. These data provide additional evidence that different classes of chemical allergen cause divergent immune responses in mice. The possibility that these characteristics may facilitate not only the identification, but also classification, of chemical allergens is discussed.

INTRODUCTION

Exposure to chemicals may induce allergic disease. A wide variety of chemicals are known to cause allergic contact dermatitis. In addition, however, some of these also have the ability to cause respiratory sensitization. An objective in this Laboratory has been to characterize the immunobiology of skin and respiratory hypersensitivity, and to investigate why some chemicals provoke respiratory allergy while others cause contact allergy exclusively.

In previous studies we have characterized immune responses induced in mice following topical exposure to trimellitic anhydride (TMA), a respiratory allergen, and to 2,4-dinitrochlorobenzene (DNCB), a potent contact allergen which appears not to possess the potential to cause respiratory sensitization (Dearman and Kimber, 1991a). Under conditions where both chemicals caused lymphocyte hyperplasia in lymph nodes draining the site of application, and each induced an IgG anti-hapten response, exposure only to TMA provoked an increase in IgE; the class of antibody which effects immediate hypersensitivity reactions characteristic of respiratory allergy (Dearman and Kimber, 1991a). These data led us to speculate that TMA and DNCB, while both immunogenic, preferentially stimulate different classes of T helper (T_H) cell. There is evidence for functional heterogeneity among murine T_H cells. Mosmann et al. (1986) have identified two populations, designated T_{H1} and T_{H2}, which differ in terms of the cytokines they secrete. Although both populations produce granulocyte/macrophage colony-stimulating factor (GM-CSF) and interleukin 3 (IL-3), only T_{H1} cells secrete

interleukin 2 (IL-2) and interferon-γ (IFN-γ), and only T_{H2} cells secrete interleukins 4, 5 and 6 (IL-4, IL-5 and IL-6) (Mosmann and Coffman, 1989). Of relevance to observations made with TMA and DNCB is the fact that the soluble products of T_{H1} and T_{H2} cells exert opposing effects on IgE production. The initiation and maintenance of IgE responses is dependent upon the availability of IL-4 (Finkelman et al., 1986; 1988a; Azuma et al., 1987). In contrast IFN-γ inhibits IgE responses (Finkelman et al., 1988b).

Our hypothesis was that TMA preferentially activates T_{H2} cells resulting in IL-4 production and IgE responses, whereas DNCB stimulates T_{H1} cells and IFN-γ production and therefore fails to provoke IgE. Support for this hypothesis derives from the fact that, in addition to influencing IgE responses, the soluble products of T_H cells also affect other immunoglobulin isotypes. It is known that IFN-γ, but not T_{H2} cytokines, promotes antibody of IgG2a isotype (Finkelman 1988b; Snapper and Paul, 1987; Stevens et al., 1988; Coffman et al., 1988). We found that, compatible with selective T_{H1} activation and IFN-γ production, DNCB provoked a significantly stronger IgG2a than IgG2b antibody response. In contrast, following exposure to TMA the ratio of IgG2a to IgG2b anti-hapten antibody was substantially less than 1 (Dearman and Kimber, 1991a). Evidence that such differential responses are not peculiar to DNCB and TMA was provided by subsequent studies performed with phthalic anhydride, a known respiratory allergen, and oxazolone a potent contact allergen. Compatible with preferential T_{H2} activation, exposure to phthalic anhydride induced an IgE response and a stronger IgG2b than IgG2a anti-hapten antibody response. Oxazolone failed to provoke IgE and induced a stronger IgG2a than IgG2b response (Dearman and Kimber, 1991b).

Taken together these data suggest that it may be possible to characterize and classify chemical allergens as a function of the immune responses they elicit in mice. In an attempt to investigate further the validity of this proposal, and to determine whether respiratory allergens other than acid anhydrides also provoke immune responses characteristic of T_{H2} activation, we have in the present study examined selected diisocyanates.

The diisocyantes studied (diphenylmethane-4,4'-diisocyanate, MDI; dicyclohexylmethane-4,4'-diisocyanate, HMDI and isophorone diisocyanate, IPDI) have each been found to have the potential to cause contact dermatitis in exposed individuals (Rothe, 1976; Emmett, 1976; Lachapelle and Lachapelle-Ketelaer, 1979). Evidence also exists that MDI and HMDI induce contact sensitization in guinea pigs (Stevens, 1967; Karol and Magreni, 1982; Stadler and Karol, 1984), and in mice (Thorne et al., 1987), and that IPDI causes contact sensitivity in mice (Stern et al., 1989). MDI is, in addition, known to cause occupational respiratory sensitization, frequently associated with the presence of specific IgE antibody (Tansar et al, 1973; Zeiss et al, 1980; Zammit-Tabona et al., 1983; Keskinen et al, 1988). Exposure of guinea pigs to MDI has also been shown to result in pulmonary hypersensitivity (Karol, 1988). In contrast, however, despite frequent industrial use and evidence for occupational contact sensitization, HMDI appears not to cause respiratory allergy (Karol, 1986). Similarly respiratory sensitization to HMDI was detected only rarely in exposed guinea pigs (Karol and Magreni, 1982). In the case of IPDI, there is, as far as we are aware, only a single documented case of suspected occupational respiratory sensitization (Clarke and Aldons, 1981).

MATERIALS AND METHODS

Animals

Young adult (8-12 weeks old) female BALB/c strain mice (Barriered Animal Breeding Unit, Alderley Park, Cheshire, UK) were used throughout these studies.

Chemicals

Diphenylmethane-4,4'-diisocyanate (MDI) was provided by ICI Polyurethanes. Dicyclohexylmethane-4,4'-diisocyanate (HMDI) and isophorone diisocyanate (IPDI) were obtained from Aldrich Chemical Co., Dorset, UK.

Measurement of lymph node cell proliferation

Groups of mice (n=4) were exposed topically on the dorsum of both ears to 25μl of various concentrations of the test chemical in 4:1 acetone:olive oil (AOO). Control mice received an equivalent volume of vehicle alone. Three days later all mice were injected intravenously via the tail vein with 20μCi of [^{3}H]methyl thymidine (specific activity 2Ci/mmol; Amersham International, Amersham, Bucks, UK) in 250μl of phosphate buffered saline (PBS). Five hours later mice were killed and the draining auricular lymph nodes excised and pooled for each experimental group. A single cell suspension was prepared by gentle mechanical disaggregation through 200-mesh stainless steel gauze. LNC were washed

twice with an excess of PBS and precipitated in 5% trichloroacetic acid (TCA). Twelve hours later pellets were resuspended with 1ml of TCA and transferred to 10ml of scintillation fluid (Optiphase MP, LKB, Flow McClean, VA). Incorporation of [^{3}H]thymidine (^{3}HTdR) was measured by β-scintillation and results expressed as the mean cpm per node for each experimental group.

Sensitization for, and elicitation of, contact sensitivity

Groups of mice (n=6) received 50μl of test chemical in AOO, or an equal volume of AOO alone, on each shaved flank. Five days following sensitization ear thickness was measured using an engineers' micrometer (Moore and Wright, Sheffield, UK). Immediately afterwards the dorsum of both ears was treated with 25μl of the challenge concentration of chemical. Elicitation reactions were measured 24hr. later as the mean percentage increase in ear thickness relative to pre-challenge values.

Sensitization for antibody

Groups of mice (n=10) received 50μl of test chemical in AOO on each shaved flank. Seven days later 25μl of the same solution diluted 1:1 with vehicle were applied to the dorsum of both ears. In one series of experiments mice (n=5) received, in the shaved nape of the neck, a single 50μl subcutaneous injection of a 0.1% solution of the test chemical in corn oil. At various periods following exposure mice were exsanguinated by cardiac puncture and serum prepared and stored at -20°C until analysis.

Hapten-protein conjugates

A 40 mol excess of chemical was added at hourly intervals to bovine serum albumin (BSA; Sigma Chemical Co., St. Louis, Mo) by stirring at room temperature for 3 hr. The pH was maintained at 8 by addition of 1M sodium hydroxide. Conjugation reactions were terminated by sequential dialysis against 0.1M sodium bicarbonate buffer (pH 8), and water. Conjugates were lyophilized and stored at -20°C. The degree of substitution was assessed using a method based upon determination of free amino groups by reaction with 2,4,6-trinitrobenzene sulfonic acid (TNBS) (Snyder and Sobocinski, 1975). Conjugates and BSA at 1mg/ml in 0.1M sodium borate buffer (pH 9.3) were incubated for 20 min. in the presence of 0.03M TNBS in borate buffer. The optical density (OD) at 420nm was measured. BSA has approximately 30 readily available binding sites per molecule, and consequently the degree of substitution (mol/mol) was calculated as follows:

$$\text{Substitution} = 1 - \frac{\text{OD sample}}{\text{OD BSA}} \times 30$$

Substitution ratios for the MDI-, HMDI- and IPDI-BSA conjugates used in this study were respectively 26:1, 16:1 and 24:1 (moles hapten : moles protein).

Enzyme-linked immunosorbent assays (ELISA)

(i) Serum IgG antibodies

IgG anti-hapten antibodies and anti-hapten antibodies of subclasses IgG2a and IgG2b were detected by ELISA. Plastic microtitre plates (Nunc Immunoplate type II, Nunc, Copenhagen, Denmark) were coated with 100μg/ml of hapten-protein conjugate in PBS by overnight incubation at 4°C. The plates were blocked by incubation for a further 30 min. at 37°C with 5% normal goat serum (NGS) in PBS. Mouse serum samples diluted 1:25 with 0.5% NGS in PBS were added to triplicate wells and incubated for 3hr. at 4°C. The plates were then incubated for 3hr. at 4°C with peroxidase-conjugated goat anti-mouse IgG (Nordic, Tilberg, The Netherlands) diluted 1:8000 with 0.5% NGS in PBS, or for 2hr. at room temperature with peroxidase-conjugated sheep anti-mouse IgG2a or IgG2b (Serotec, Kidlington, UK) diluted 1:2000 or 1:4000 respectively, in 0.5% NGS in PBS. Enzyme substrate (o-phenylenediamine and urea hydrogen peroxide) was added and the reaction stopped by addition of 0.5M citric acid after either 5 min. (IgG2a, IgG2b) or 15 min. (IgG). Substrate conversion was measured using an automated reader (Multiskan, Flow Laboratories, Irvine, Ayrshire, UK). Between each incubation, plates were washed with PBS containing 0.05% Tween 20. Antibody titres were expressed as mean OD at 450 nm, corrected for background staining recorded with normal mouse serum at an equivalent concentration.

(ii) Total serum IgE

Serum IgE was measured using a sandwich ELISA. Plastic microtitre plates were coated with 2.5μg/ml rat monoclonal anti-mouse IgE antibody BIE3 (kindly provided by Dr D. H. Conrad, Johns Hopkins University School of Medicine, Baltimore MD.) in 0.1M carbonate buffer (pH 9.6) by overnight incubation at 4°C. The plates were then blocked by treatment for 30 min. at 37°C with 5% NGS in PBS. Test serum samples and a mouse monoclonal IgE anti-dinitrophenol (DNP; clone SPE-7; ICN Immunobiologicals, Bucks, UK) diluted to various extents in 0.5% NGS in PBS were added to triplicate wells and the plates incubated for 3hr. at 4°C. Plates were then incubated at 4°C for 2hr. with 100ng/ml biotin-conjugated rat anti-mouse IgE (Serotec) and for a further 50 min. at room temperature with a 1:1000 dilution of streptavidin-horse radish peroxidase conjugate (Serotec). Substrate (as detailed above) was added and the reaction terminated after 15 min. by addition of 0.5M citric acid. Between each incubation the plates were washed with PBS containing 0.05% Tween 20. Optical density at 450 nm was measured as described above. The concentration of IgE in test serum samples was derived from a standard curve for monoclonal mouse IgE. Results are expressed as serum IgE concentration (μg/ml).

RESULTS

Lymphocyte proliferative responses and contact sensitization

Lymphocyte proliferative responses in draining lymph nodes were measured 3 days following exposure of mice to various concentrations of MDI, HMDI or IPDI. As the results recorded in Table 1 demonstrate, exposure to each of these chemicals caused a concentration-related increase in LNC proliferation. In previous studies in this Laboratory we have found that induced lymphoproliferative responses in draining lymph nodes correlate with, and provide a useful marker of, contact sensitization potential (Kimber and Weisenberger, 1989; Kimber and Dearman, 1991). Accordingly, MDI, HMDI and IPDI were each found to induce contact sensitivity in mice. Animals were sensitized on each shaved flank with 50μl of various concentrations of test chemical, and five days later elicitation reactions measured as a function of challenge-induced increases in ear thickness. The results illustrated in Figure 1 reveal that similar contact sensitization was observed with each chemical, with IPDI exhibiting possibly the greatest activity. Maximum sensitization to HMDI was achieved with 1% of the chemical, higher concentrations inducing reduced activity, possibly as a result of local toxicity.

Taken together, the conclusion that can be drawn from these data is that, on a weight/volume basis, these chemicals exhibit comparable activity with regard to skin sensitization and LNC proliferation in mice.

Antibody responses

Mice received 50μl of either 2% or 1% of the test chemical in AOO bilaterally on the shaved flanks. Seven days later ears were painted with 25μl of a 1:1 dilution of the same solution (1% or 0.5%). At various times following the initiation of exposure mice were exsanguinated and the presence of serum antibody measured by ELISA. Contrary to expectations, exposure of mice to HMDI or IPDI, at concentrations which caused LNC proliferation and contact sensitization, failed to provoke a measurable IgG anti-hapten antibody response (Figure 2). In contrast, a significant response was observed following exposure to MDI. A clear indication of IgG anti-hapten antibody was first recorded 14 days after the initiation of exposure to 1% followed by 0.5% MDI. IgG antibody after treatment with 2% followed by 1% MDI was found at 8 days, with the response nearly maximal at 14 days (Figure 2).

Naturally the possibility can not be excluded that topical exposure to higher concentrations of IPDI and HMDI might have resulted in the appearance of specific IgG antibody. Such experiments were not attempted as concentrations in excess of 2% and 5% IPDI and HMDI respectively proved toxic.

Support for a clear difference between the ability of MDI and the ability of HMDI and IPDI to stimulate antibody responses derives from subsequent experiments in which specific IgG was measured following subcutaneous injection of chemical. Mice received 50μl of a 0.1% solution of chemical in corn oil and serum examined for the presence of IgG anti-hapten

antibody 21 days later. A reponse was observed only with MDI; HMDI and IPDI failed to provoke specific IgG antibody (Figure 3). In the case of HMDI at least, it was possible to confirm that the BSA-HMDI conjugate used as antigen in the ELISA was appropriate for measurement of antibody induced by this chemical. In a separate study in this Laboratory it was found that anti-HMDI antibody, reactive with the conjugate used here, could be provoked by intradermal immunization of guinea pigs to HMDI (data not presented).

We have reported previously that the respiratory allergens TMA and phthalic anhydride induce considerably stronger IgG2b than IgG2a anti-hapten antibody responses (Dearman and Kimber, 1991a; b). Using isotype-specific ELISA this was found also to be the case with MDI. The data summarized in Figure 4 demonstrate that, at each time point examined following exposure to MDI, there was substantially more IgG2b than IgG2a specific antibody present. The results shown in Figure 4 represent mean values derived in each case from groups of 10 mice. A remarkable feature of these experiments was that at all time points, the ratio of IgG2b:IgG2a anti-MDI antibody was invariably considerably greater than 1 in each individual serum isolated from exposed mice (Table 2).

Finally, we sought to determine whether exposure resulted in changes in the serum concentration of IgE. Previous studies have shown that treatment of mice with TMA or phthalic anhydride was associated with a significant elevation in IgE; under the same conditions of exposure no increase was observed with either DNCB or oxazolone (Dearman and Kimber, 1991a; b). The serum IgE concentration of untreated BALB/c mice used in this study

was found to vary between 0.1 and 0.3μg/ml. Such values are in broad agreement with data reported by Azuma et al. (1987). Topical exposure of mice to HMDI or IPDI failed to cause an elevation in the concentration of serum IgE. In marked contrast, exposure to MDI resulted in a substantial increase in IgE. At all time points examined the serum concentration exceeded 1μg/ml, reaching nearly 1.5μg/ml 14 days following the initiation of exposure (Figure 5).

DISCUSSION

Our working hypothesis, based upon previous studies and reported elsewhere, is that chemicals which have the potential to induce respiratory sensitization preferentially stimulate T_{H2} cells. Chemicals which appear not to cause respiratory allergy, but which are nevertheless contact sensitizers, are thought to preferentially activate T_{H1} cells (Dearman and Kimber, 199a; b). Respiratory allergy is, in most cases, associated with the appearance of IgE antibody which effects immediate hypersensitivity reactions. A preferential activation of T_{H2} cells by chemicals such as TMA and phthalic anhydride, resulting in the production of IL-4 and consequently the promotion of IgE responses, would be compatible with the ability of such agents to cause respiratory allergy. In the present study the known human respiratory sensitizer MDI was found to elicit antibody responses in mice equivalent to those observed previously following exposure to TMA and phthalic anhydride (Dearman and Kimber, 199a; b). Thus, MDI caused an increase in the serum concentration of IgE. and a preferential IgG2b rather than IgG2a response. Moreover, exposure to MDI, in common with TMA and phthalic anhydride was found, on the basis of passive cutaneous anaphylaxis assays, to induce hapten-specific IgE antibody (data not presented). One conclusion that can be drawn is that, with respect to chemical respiratory allergens, the induction of murine immune responses characteristic of T_{H2} cell activation is not restricted to acid anhydrides. It must be emphasized that it is currently our view that respiratory sensitizers such as MDI

cause a preferential, rather than exclusive, stimulation of T_{H2} cells. Such would accommodate the fact that MDI, and other respiratory sensitizers so far examined, are also able to induce contact allergy. The production of IgE is reciprocally regulated by the products of activated T_{H1} and T_{H2} cells. Interferon γ inhibits, and IL-4 promotes, IgE antibody (Snapper and Paul 1987; Finkelman et al., 1988a; b). A preferential activation of T_{H2} cells would presumably result in a balance of these cytokines permissive for IgE production without necessarily compromising the development of contact sensitization via the action of T_{H1} cells. In the context of reciprocal expression of respiratory and contact allergy it is of interest that a study of diisocyanates reported by Thorne et al. (1987) provided some evidence for an inverse relationship between the potentials for contact and respiratory sensitization.

Our previous investigations have indicated that exposure of mice to contact allergens which apparently lack the capacity for respiratory sensitization results in a preferential activation of T_{H1} cells. Interferon γ, a T_{H1} cell product, inhibits IgE but promotes IgG2a antibody production (Snapper and Paul, 1987; Finkelman et al., 1988b; Stevens et al., 1988; Coffman et al., 1988). DNCB and oxazolone failed to induce IgE antibody, and provoked a considerably stronger IgG2a than IgG2b anti-hapten response (Dearman and Kimber, 1991a; b).

In the present study we chose to compare with MDI, the diisocyanates HMDI and IPDI. The available evidence suggests that HMDI and IPDI are unable to induce respiratory allergy, or at least do so very rarely. Banks et al. (1986) conclude that the ability of these two diisocyanates to cause respiratory illness is weak.

The unexpected finding was that, under the conditions of exposure used, neither chemical provoked a measurable antibody response of any class. In the absence of IgG it was not possible to analyse the isotype distribution of antibody responses. Pertinent to these observations is a study reported by Karol and Magreni (1982) in which it was found that topical exposure of guinea pigs to HMDI resulted in the development of contact sensitivity, provoked little if any antibody, and failed to induce pulmonary sensitivity.

There can be no doubt, however, that in the present study both HMDI and IPDI were immunogenic, causing a vigorous lymphocyte proliferative response in draining lymph nodes and contact sensitization. Even though it is not possible to conclude that these chemicals will be unable in all circumstances to provoke an antibody response in mice, the data indicate clearly that cell-mediated immune responses are preferentially induced. It is consequently tempting to speculate that in the case of HMDI and IPDI there is an even more selective activation of T_{H1} cells. It is clear that while the primary function of T_{H2} cells is to promote and regulate antibody responses, T_{H1} cells execute cell-mediated immune responses, of which delayed-type hypersensitivity (DTH) is one example. T_{H1}, but not T_{H2} cells will transfer DTH reactions (Cher and Mosmann, 1987). Moreover, IFN-γ, a product of T_{H1} cells, has been found to play an important role in the development of DTH (Diamantstein et al., 1988; Fong and Mosmann, 1989).

If, as we speculate, respiratory and contact chemical sensitizers preferentially induce different functional populations of T_H cells

in mice, it is relevant to question the significance of this with respect to human allergic disease. Clear evidence for the existence of such a well-defined functional heterogeneity among human T_H cells is unavailable. Although human T_{H1} and T_{H2} cells can be demonstrated (Umetsu et al., 1988) many T cell clones secrete both T_{H1}- and T_{H2}-type cytokines (Paliard et al., 1988). It has been found that immature murine T_H cells may produce both classes of cytokine, the suggestion being that while murine T helper cells readily differentiate into one or other functional subpopulation, human T_H cells exhibit greater stability as mixed cytokine producers (Mosmann and Coffman, 1989). There is no doubt that although a well-defined functional dichotomy among T_H cells is more difficult to demonstrate in man, human IgE responses in vitro are reciprocally regulated by IL-4 and IFN-γ (Del Prete et al., 1988; Pene et al., 1988; Romagnani et al., 1989; Chretien et al., 1990). Moreover, evidence is emerging that, as in the mouse, T_{H1}- and T_{H2}-type cytokines influence not only IgE responses but also IgG isotype production. IL-4, for instance, in addition to promoting IgE production also induces IgG4 synthesis by human lymphocytes; an effect which is inhibited in a dose-dependent manner by IFN-γ (Ishizaka et al., 1990.

Finally, it is necessary to consider the opportunities these observations may provide for the identification and classification of chemical allergens in the context of predictive toxicology. Karol and her colleagues have been instrumental in developing guinea pig models of respiratory sensitization to chemicals (Karol et al., 1978; 1980; 1981; Karol, 1988). Animals sensitized by inhalation exposure to the free or protein-bound

chemical, or by dermal immunization, were shown to exhibit symptoms of respiratory distress and pulmonary hypersensitivity following subsequent inhalation challenge, usually with a hapten-protein conjugate. Recently significant changes in respiratory rate have been reported following inhalation challenge with free (unconjugated) TMA of guinea pigs previously sensitized to the same chemical by intradermal injection (Botham et al., 1989). Although such models are valuable, particularly in the context of analyzing the elicitation of pulmonary hypersensitivity, they do not necessarily lend themselves to the routine predictive assessment of respiratory sensitization potential. On the basis of the data presented here and previous studies, we believe that it may be possible to characterize and classify chemical allergens as a function of the nature of immune responses induced in mice. All known respiratory allergens tested to date (TMA, phthalic anhydride and MDI) provoke an increase in IgE and considerably more IgG2b than IgG2a antibody. In contrast chemicals which lack, or have only very weak, potential for respiratory sensitization, but which are nonetheless contact allergens, appear to fall into 1 of 2 categories. Either they fail to induce IgE, but elicit a stronger IgG2a than IgG2b response (DNCB and oxazolone), or alternatively they fail to provoke antibody altogether (HMDI and IPDI). With the benefit of additional studies it may be possible to make use of these characteristics to facilitate not only identification of chemical allergens, but also accurate prediction of the form chemically-induced allergic reactions may take.

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TABLE 1

DRAINING LNC PROLIFERATION FOLLOWING TOPICAL EXPOSURE TO MDI, HMDI AND IPDI

Concentration (% w/v)	Draining LNC proliferation. ^{3}HTdR incorporation mean cpm/node x 10^{-2} (incremental increase relative to control)		
	MDI	HMDI	IPDI
0	0.89	0.73	0.67
0.05	-	-	1.21 (1.81)
0.1	1.37 (1.54)	0.95 (1.30)	2.94 (4.39)
0.25	2.14 (2.40)	3.15 (4.32)	15.55 (23.21)
0.5	6.15 (6.91)	9.08 (12.44)	20.49 (30.58)
1	24.07 (27.04)	52.23 (71.55)	30.93 (46.16)
2.5	39.30 (44.16)	66.30 (90.82)	36.79 (54.91)
5	46.50 (52.25)	69.54 (95.26)	-
10	75.32 (84.63)	-	-

Groups of mice (n=4) received 25μl of various concentrations of test chemical, or an equal volume of vehicle (AOO) alone on the dorsum of both ears. Three days later all mice received 250μl of PBS containing 20μCi of [^{3}H]methyl thymidine iv via the tail vein. Mice were killed 5 hr. later and draining auricular lymph nodes isolated and pooled for each experimental group. Results are expressed as mean cpm/node. In parentheses are shown incremental increases in ^{3}HTdR incorporation relative to vehicle-treated controls.

TABLE 2

RATIO OF IgG2a:IgG2b ANTI-HAPTEN ANTIBODY IN SERA OF INDIVIDUAL MICE EXPOSED TO MDI

IgG2a:IgG2b anti-hapten antibody Days following the initiation of exposure		
8	14	21
1 : 9.83	1 : 9.89	1 : 7.51
1 : 8.25	1 : 5.43	1 : 6.81
1 : 5.18	1 : 4.95	1 : 6.11
1 : 5.06	1 : 4.85	1 : 5.77
1 : 4.59	1 : 4.03	1 : 4.59
1 : 3.98	1 : 3.47	1 : 3.76
1 : 3.91	1 : 3.38	1 : 3.49
1 : 3.38	1 : 3.15	1 : 2.98
1 : 3.37	1 : 3.00	1 : 2.35
1 : 2.50	1 : 1.84	1 : 2.04
Mean = 1 : 4.99	Mean = 1 : 4.40	Mean = 1 : 4.54

Groups of mice (n=10) received 50μl of 2% MDI on each shaved flank. Seven days later all mice received 25μl of the same chemical (1%) on the dorsum of both ears. Mice were exsanguinated 8, 14 or 21 days following the initiation of exposure. The presence of IgG2a and IgG2b anti-hapten antibody was measured by isotype-specific ELISA. The ratio of IgG2a:IgG2b antibody obtained with sera from individual mice, together with mean values, are shown.

FIGURE LEGENDS

Figure 1

Contact sensitization of mice to IPDI (a), HMDI (b) and MDI (c). Groups of mice (n=6) received 50μl of various concentrations of the test chemical in AOO, or an equal volume of AOO alone, bilaterally on the shaved flanks. Five days later the ear thickness of all mice was measured immediately prior to challenge of the dorsum of both ears with 25μl of 0.5% of the relevant chemical. Ear thickness was re-evaluated 24hr. later and elicitation reactions recorded as the percentage increase in ear thickness relative to pre-challenge values. Mean values are illustrated and, where greater than 5%, standard error.

Figure 2

IgG anti-hapten antibody response following topical exposure to IPDI (a), HMDI (b) and MDI (c). Groups of mice (n=10) received 50μl of the test chemical in AOO on each shaved flank (● 2%, ■ 1%). Seven days later mice received 25μl of a 1:1 dilution of the same solution on the dorsum of both ears. At various periods following the initiation of exposure (8, 14 and 21 days) mice were exsanguinated and individual sera analysed for the presence of IgG anti-hapten antibody by ELISA. Mean values are illustrated, and where greater than 5%, standard error.

Figure 3

IgG anti-hapten antibody response following subcutaneous exposure of mice to IPDI, HMDI and MDI. Groups of mice (n=5) received a single subcutaneous injection of a 0.1% solution of the test chemical in corn oil. Twenty-one days later mice were exsanguinated and individual sera analysed for the presence of IgG anti-hapten antibody by ELISA. Mean values ± SE are illustrated.

Figure 4

IgG2a (●) and IgG2b (■) anti-hapten antibody response following topical exposure of mice to MDI. Mice (n=10) received 50μl of 1% MDI in AOO on each shaved flank. Seven days later all mice received 25μl of the same chemical on the dorsum of both ears. At various periods following the initiation of exposure (8, 14 and 21 days) mice were exsanguinated and the presence of IgG2a and IgG2b anti-hapten antibody in individual sera analysed by isotype-specific ELISA. Mean values are illustrated and, where greater than 5%, standard error.

Figure 5

Serum IgE concentration following topical administration of IPDI (■), HMDI (●) and MDI (○). Groups of mice received 50μl of 2% of the test chemical in AOO bilaterally on the shaved flanks. Seven days later all mice received 25μl of a 1:1 solution of the same chemical on the dorsum of both ears. At various periods following the initiation of exposure (8, 14 and 21 days) mice were exsanguinated and the concentration of IgE in individual sera measured by double sandwich ELISA. Results are recorded as the mean concentration of IgE ± SE (μg/ml).

Figure 1

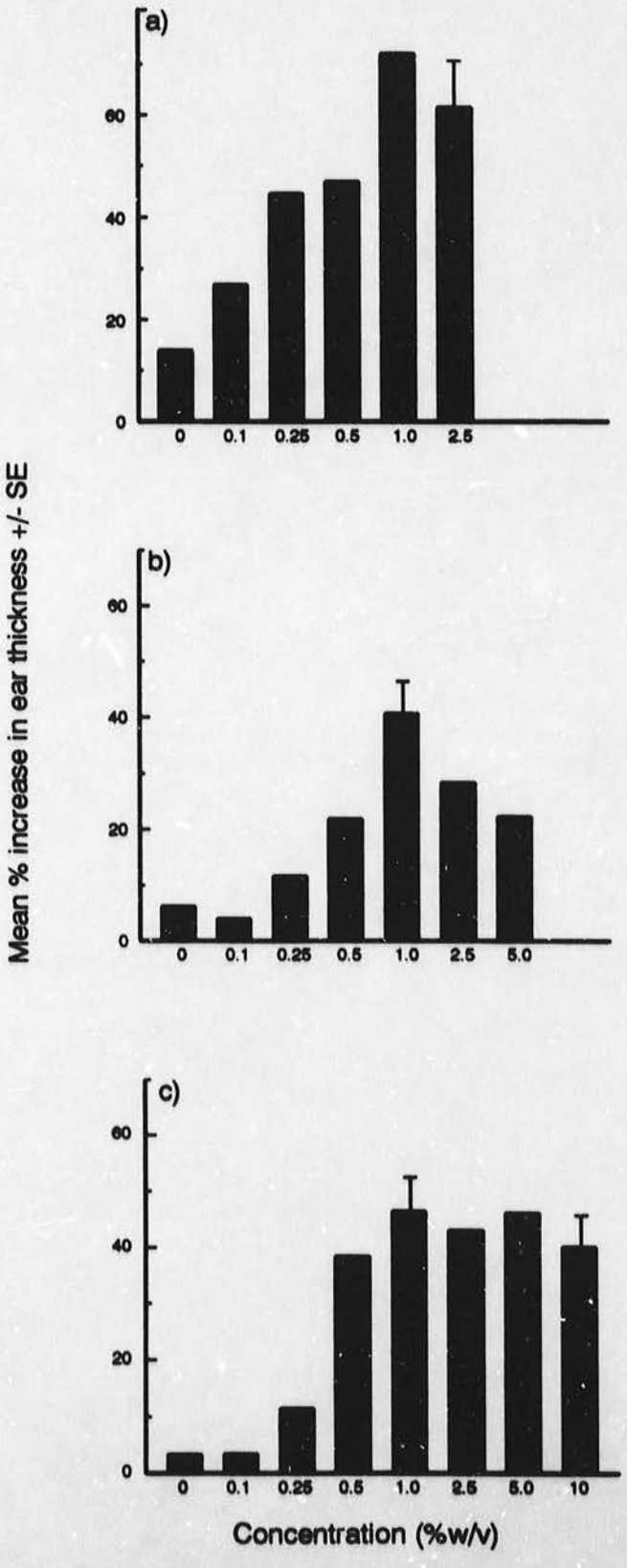

a)
b)
c)
Mean % increase in ear thickness +/- SE
0
20
40
60
0 0.1 0.25 0.5 1.0 2.5
0 0.1 0.25 0.5 1.0 2.5 5.0
0 0.1 0.25 0.5 1.0 2.5 5.0 10
Concentration (%w/v)

Figure 2.

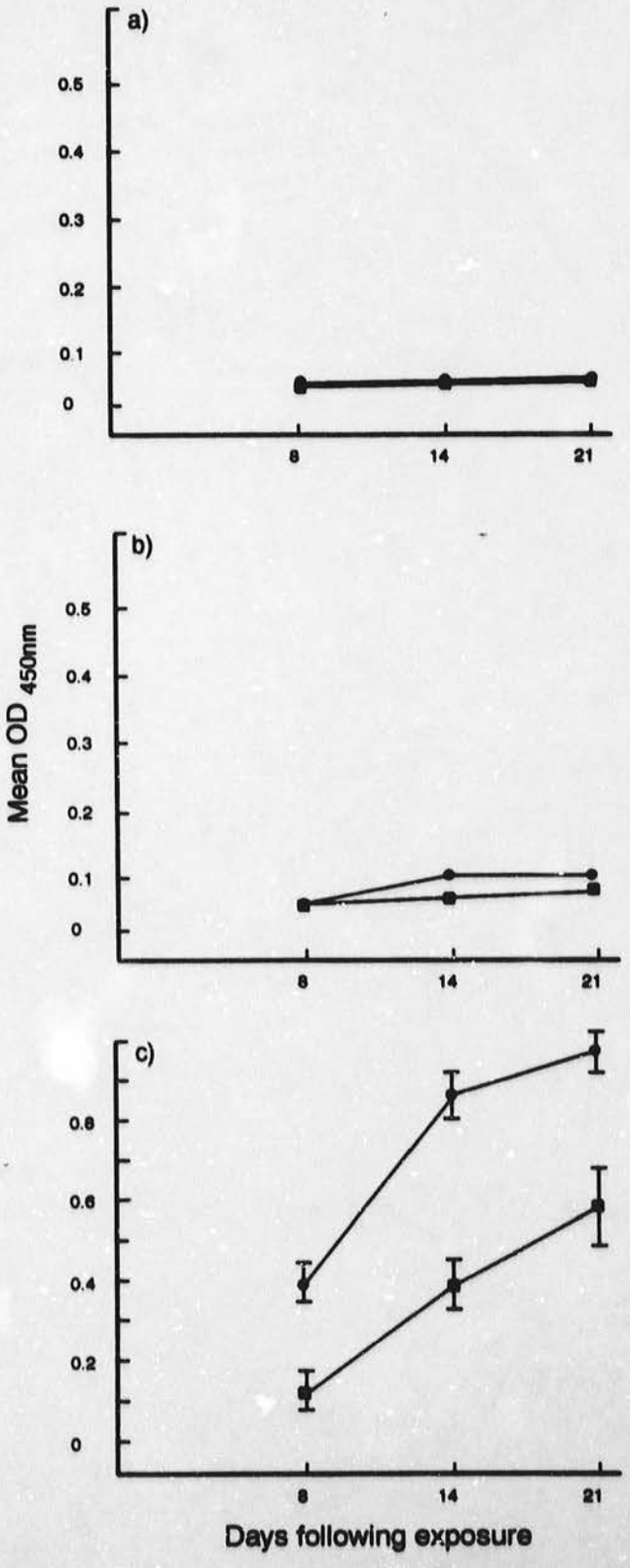

a)
b)
c)
Mean OD 450nm
0.5
0.4
0.3
0.2
0.1
0
0.8
0.6
8
14
21
Days following exposure

Figure B

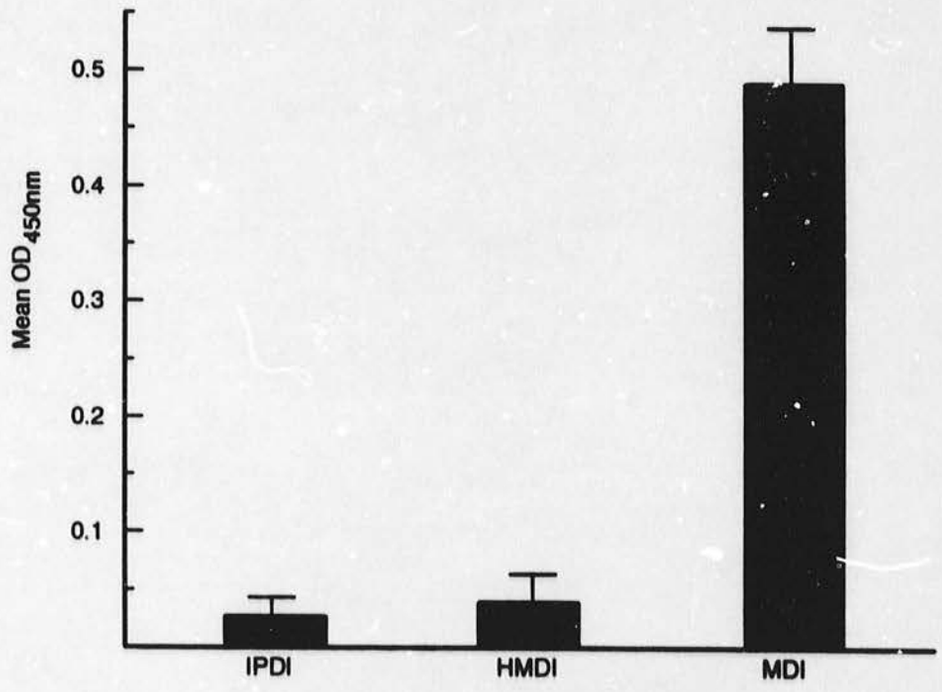


Figure 4

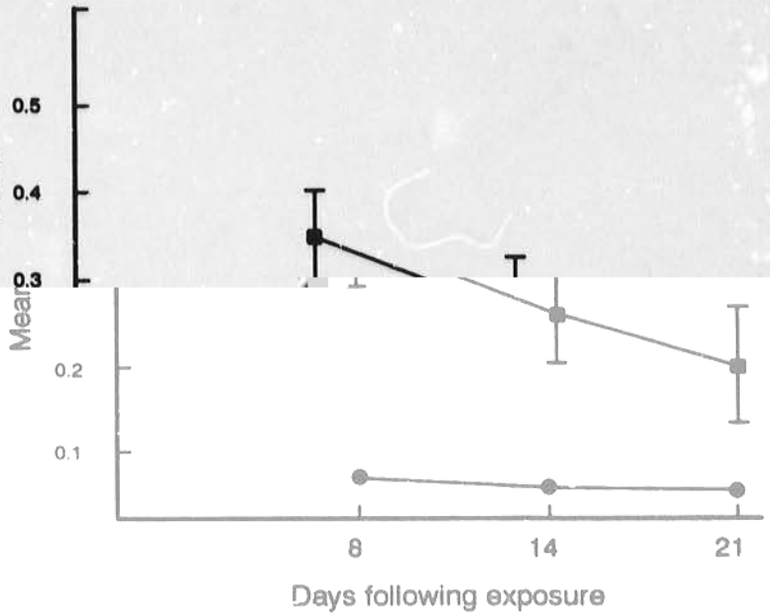
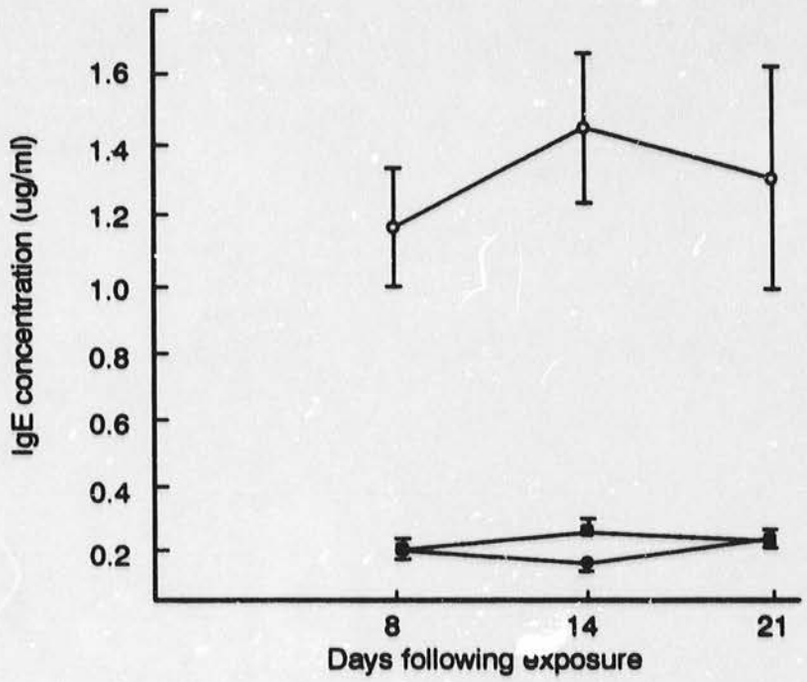


Figure 5


IgE concentration (ug/ml)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
8
14
21
Days following exposure

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